Rickettsia Pays the Piper; New Actors and Some Bad Actin'

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Pathogens such as *Listeria* have been used to define host factors regulating actin dynamics, including the Arp2/3 complex. In this issue of *Cell Host & Microbe*, Serio et al. (2010) use *Rickettsia* to identify a new complex, based on profilin, which regulates actin dynamics in normal cells and which may be exploitable by diverse pathogens.

Bacterial and viral pathogens exploit the host cytoskeleton to move into, through, and out of infected cells, and some pathogens even stimulate the cells they infect to themselves move. As such, understanding how pathogens usurp host cytoskeletal function has proven tremendously valuable for elucidating basic mechanisms of both pathogen dissemination and cytoskeletal function. It has long been recognized that actin monomers spontaneously polymerize at very low rates to form filaments; thus, a "nucleator" composed of a few monomers is required to initiate polymerization. How does this happen? Attempts to purify actin polymerizing agents yielded numerous actin-binding proteins but no clear mechanism—that is, until Welch, Mitchison, and colleagues turned to a pathogen *Listeria monocytogenes*, which polymerizes actin as a means of moving through the cytoplasm of an infected cell and burrowing into an apposing uninfected cell. Using *Listeria* motility in a cell-free extract system as an assay (Theriot et al., 1994), Welch, Mitchison, and colleagues purified a complex of seven proteins, called the Arp2/3 complex, which was required for not only *Listeria* motility (Welch et al., 1997b) but also for actin polymerization at the leading edge of motile cells (Welch et al., 1997a). The Arp2/3 complex had everything required, including two actin-related proteins that served as nucleators for nascent filaments. Welch and Mitchison went on to demonstrate that the *Listeria* surface protein ActA was the bacterial activator of the Arp2/3 complex (Welch et al., 1998).

But what is the cellular homolog of ActA? Using a two-hybrid approach, Machesky and Insall found that the Arp2/3 complex bound to SCAR, a mammalian homolog of an actin motility protein identified genetically in the amoeba *Dictyostelium discoidium*, and WASP (Machesky and Insall, 1998). Kirschner and colleagues, using biochemical approaches, demonstrated that N-WASP activates the Arp2/3 complex using a C-terminal domain that resembled ActA (Rohatgi et al., 1999). Simultaneously, work with enteropathogenic *Escherichia coli* (EPEC) showed that the C-terminal domain of N-WASP was required in cells to form actin-filled membraneous protrusions, or pedestals, beneath attached bacteria, and thus might serve as a signaling bridge between molecules at the plasma membrane and the actin polymerization machinery (Kalman et al., 1999). Work with poxviruses, which form actin protrusions upon their emergence at the cell surface, and *Shigella flexneri*, which, like *Listeria*, move through the cytoplasm, solidified a role for the N-WASP-Arp2/3 system in pathogen motility.

But there were heretical rumbles as other actin polymerizing proteins were identified. Was Arp2/3 the only means to polymerize actin, and do all pathogens use Arp2/3 complex for motility? In this issue of *Cell Host & Microbe*, Serio et al. (2010) define factors required for motility of *Rickettsia* species. *Rickettsia* are Gram-negative intracellular bacteria that are disseminated by ticks and cause Rocky Mountain spotted fever and typhus. Like *Listeria*, *Rickettsia* escape from a phagosomal compartment into the cytosol, where they use actin to propel themselves within and between cells. Although *Rickettsia* have a protein called RickA that can nucleate actin via the Arp2/3 complex in vitro, not all *Rickettsia spp.* that move express it. The question asked by Serio et al. was whether some other system might be at play.

Using an RNAi cytoskeletal sublibrary in *Drosophila* S2 cells, Serio et al. identify four proteins: fimbrin, capping protein and cofilin, and profilin, but not the Arp2/3 complex and other proteins utilized by other pathogens. Moreover, they go on to define similar roles of these proteins in mammalian cells. All of these proteins are known to regulate actin filament dynamics and organization. Fimbrin crosslinks actin filaments and forms tight actin bundles, capping protein caps the barbed ends of actin filaments, and inhibits polymerization and depolymerization, and cofilin severs and depolymerizes actin filaments. Indeed, Pollard and Borisy (2003) proposed that profilin, capping protein, and cofilin regulate Arp2/3-dependent "dendritic actin nucleation" in lamellipodial actin networks and *Listeria* actin tails.

How might these factors coordinate actin dynamics in the absence of Arp2/3 complex? The key may be profilin, which catalyzes exchange of actin-bound ATP/ADP to produce polymerization-competent ATP-actin momomers and promotes elongation from exposed barbed ends of actin filaments, but not from pointed ends. Therefore, when barbed ends are capped by capping protein, profilin sequesters actin monomers that are only available for polymerization at new actin nucleation sites. Serio et al. (2010) propose that capping protein caps old actin barbed ends away from the bacterial surface so that profilin-actin is directed to polymerize actin and elongate the filament at the bacterial surface.

Profilin by itself inhibits actin nucleation and therefore would need to cooperate

with a nucleating factor(s) to promote actin motility. It is known that profilin and formins interact in such a manner. Thus, profilin-actin complexes may bind to the formin-homology (FH) domain of formin, which can in turn nucleate actin polymerization and bind to barbed ends processively as the filaments grow. Formin-profilin complexes can thereby cooperatively enhance filament elongation at barbed ends. However, Serio et al. (2010) tested six *Drosophila* formins, but none was required for *Rickettsia* motility, though it remains possible that these proteins play a redundant role. Alternatively, in a manner analogous to ActA in *Listeria*, the bacteria may itself encode a formin-like protein. In this regard, *R. rickettsii Sca2* is required for motility and virulence (Kleba et al., 2010); it will be interesting to see whether the protein encoded by this gene contains an FH-like domain that interacts with profilin.

Using *Rickettsia*, Serio et al. (2010) have revealed the function of a set of cytoskeletal proteins whose role in actin dynamics have been suspected but never so clearly defined. With *Rickettsia*, it will be interesting to see whether the RickA-Arp2/3 or Sca2-profilin complexes catalyze distinct actin-dependent processes during infection. Indeed, it may turn out that many pathogens use both actin polymerization systems. Such explorations may, in turn, elucidate novel regulatory signaling mechanisms as well as an understanding of how normal cells control actin dynamics.

REFERENCES

Kalman, D., Weiner, O.D., Goosney, D.L., Sedat, J.W., Finlay, B.B., Abo, A., and Bishop, J.M. (1999). Nat. Cell Biol. *1*, 389–391.

Kleba, B., Clark, T.R., Lutter, E.I., Ellison, D.W., and Hackstadt, T. (2010). Infect. Immun. *78*, 2240–2247.

Machesky, L., and Insall, R.H. (1998). Curr. Biol. *8*, 1347–1356.

Pollard, T.D., and Borisy, G.G. (2003). Cell *112*, 453–465.

Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., Takenawa, T., and Kirschner, M.W. (1999). Cell *97*, 221–231.

Serio, A.W., Jeng, R.J., Haglund, C.M., Reed, S.C., and Welch, M.D. (2010). Cell Host Microbe *7*, this issue, 388–398.

Theriot, J.A., Rosenblatt, J., Portnoy, D.A., Goldschmidt-Clermont, P.J., and Mitchison, T.J. (1994). Cell *76*, 505–517.

Welch, M.D., Depace, A.H., Verma, S., Iwamatsu, A., and Mitchison, T.J. (1997a). J. Cell Biol. *138*, 375–384.

Welch, M.D., Iwamatsu, A., and Mitchison, T.J. (1997b). Nature *385*, 265–269.

Welch, M.D., Rosenblatt, J., Skoble, J., Portnoy, D.A., and Mitchison, T.J. (1998). Science *281*, 105–108.

A Call to Arms: Interferons Prepare Bone Marrow Cells to Battle Peripheral Infections

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Viral infection leads to the rapid production of type I interferons within infected tissues. In this issue of *Cell Host & Microbe*, Hermesh et al. (2010) demonstrate that interferons produced following respiratory viral infection program leukocytes in the bone marrow to resist infection before trafficking to the lung.

Immune responses to viral infection of the respiratory tract are triggered by the innate recognition of pathogen-associated molecular patterns. One of the earliest antiviral responses is the synthesis of type I interferons (IFNs), which can signal back to the infected cell, as well as to surrounding cells, through the ubiquitously expressed IFNα/β receptor. Signaling through the IFNα/β receptor initiates a positive feedback loop, resulting in enhanced IFN production, the expression of genes that inhibit viral replication, and the amplification of the antiviral response (Moltedo et al., 2009). Concomitantly, a robust inflammatory response, including the production of cytokines and chemokines, serves to recruit leukocytes to the lung. Hermesh and colleagues now show that type I IFNs produced in the lung can interact directly with leukocytes residing in the bone marrow, activating antiviral transcription programs that protect these cells from infection with a wide range of viruses (Hermesh et al., 2010). Taken in broader context, these findings demonstrate that peripheral infections can "communicate" with leukocytes in the bone marrow, priming these cells to resist infection prior to their arrival in infected tissues, thus promoting their survival and assuring their ability to carry out antiviral functions (Figure 1).

The ability of type I IFNs to induce antiviral gene expression and inhibit viral replication within infected tissues has been well described (Katze et al., 2002). However, previous models for the effect of type I INFs on migrating leukocytes during peripheral infections assumed that IFNα/β receptor signaling, and expression of IFN-inducible genes, occurred once leukocytes migrated to infected tissues. Hermesh et al. (2010) used